Detection of Proteins in Electrophoresis

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Detection Techniques: Staining, Autoradiography and Blotting

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Introduction

Polyacrylamide gel electrophoresis (PAGE) is a highly reliable and widely used technique for the separation, identification and characterization of proteins and protein mixtures. Although two-dimensional (2D)-PAGE, which combines protein isoelectric focusing (IEF) in the first dimension with sodium dodecyl sulfate (SDS)-PAGE molecular sieving in the second dimension, provides the highest resolution allowing one to separate 1000–2000 individual polypeptide spots on a single gel, 2D-PAGE is technically very demanding. However, in the vast majority of applications, one-dimensional (1D)-PAGE, specifically SDS-PAGE, provides sufficient resolution and is especially well suited for the simultaneous analysis of multiple protein samples on a single gel. Since its introduction in 1951, very few modifications to the basic protocols for preparing and running 1D-PAGE gels have been made, although considerable advances have been introduced for the detection and analysis of PAGE separated proteins.

Post-electrophoretic gel staining is the most frequently used method for the detection of individual protein bands or spots on 1D- and 2D-PAGE gels, respectively, although procedures for prestaining proteins prior to PAGE have been described. Detection is usually performed either *in situ* within the polyacrylamide gel matrix itself or following Western electroblot transfer of proteins from PAGE gels on to polymeric membrane support matrixes. Detection systems include organic dye and metal salt-based staining protocols, fluorescent group tagging, specific protein–ligand/receptor interactions, enzymic activity detection, as well as group-specific (e.g. glyco-, phospho-, lipoproteins, etc.) staining and immunological detection of antibody–antigen complexes. Alternatively, proteins which have been labelled with radioactive molecules, either prior to or post-electrophoretically, can be visualized using autoradiographic and fluorographic detection on X-ray film. Although a plethora of protein staining and visualization protocols have been described, none is totally ideal and often the use of multiple protein staining and/or protein labelling procedures is necessary.

Protein Staining

Organic Dyes

Many of the organic dyes and stains that have been adopted for the detection of proteins in polyacrylamide gels and on membranes have been derived from dyes originally developed for the textile industry. Currently the most commonly used dyes include Amido Black, Procion Blue RS, Ponceau S, Alcian Blue, Fast Green FCF, Coomassie[1,2] Brilliant Blue R-250 (R = reddish hue) (CBB-R) and Xylene Cyanine Brilliant G (confusingly referred to as Coomassie Brilliant Blue G-250) (G = greenish hue) (CBB-G). Recently, inorganic metal ion-based staining procedures have been developed that provide highly sensitive methods of protein visualization.

CBB-R and CBB-G are the most sensitive, convenient and economical to use of the commonly available dyes, and have become the reagents of choice for protein staining. CBB-R is a nonpolar, sulfated aromatic dye that is generally used in methanolic acetic acid solutions where excess CBB is removed from the gel matrix by destaining. An acidic environment is required for optimal CBB staining to enhance ionic interactions between the dye molecules and basic amino groups of the protein as well as to augment dye–protein interactions due to hydrogen bonding, van der Waals attraction and hydrophobic bonding.

[1] Any reference to a trademark or proprietary product does not constitute endorsement of that product by the US government and does not imply its approval to the exclusion of other products.

[2] Coomassie™ is a registered trademark held by the Imperial Chemical Industries (ICI). Equivalent CBB dyes under their own trademarks include Serva Blau (Blue) R or G, PAGE blue 83 (R) or 90 (G), Kenacid blue R, Supranocyanin 6B or G, Brilliant Blue R and Microme no. 1137.

Typically, CBB staining is performed using a 0.1–0.2% CBB-R (w/v) in aqueous (v/v) 45% methanol and 10% acetic acid. The duration of staining is dependent on gel thickness and polyacrylamide composition and destaining is performed using either passive diffusion or electrophoretic destaining. After destaining, gels can be stored in 7.5% acetic acid in which the dye–protein complex is fixed and the colour is relatively stable.

An alternative CBB-based procedure exhibiting very low background staining has been described using CBB-G. Incubation of gels in a colloidal suspension of CBB-G in aqueous trichloroacetic acid (TCA) results in the formation of dye–protein complexes, in which CBB-G interacts with proteins only at the surface of the gels and does not penetrate into the gel matrix, thus minimizing background staining. Major protein bands are visible within 5–10 min and for optimal staining of less abundant proteins gels should be left in the staining solutions for several hours to overnight followed by destaining in 5% TCA.

Amido Black (Buffalo black NBR, naphthalene black 12B, aniline blue black, napththol blue black, acid black 1 and amido schwartz) was probably the first dye used to stain proteins in polyacrylamide gels; however, its use today is less frequent because of the availability of more sensitive CBB-based protocols. None the less, Amido Black still enjoys numerous applications because of its rapid staining and destaining properties.

A dye especially well suited for quantitation applications is Fast Green FCF (food green 3). Fast Green exhibits a greater linearity of staining as compared to CBB-R and also has the capacity to form stable coloured complexes with histones, thereby making it a useful group-specific stain. In contrast to CBB, Fast Green does not bind to carrier ampholytes and can be used for staining of proteins in IEF gels.

Recently, protein staining procedures utilizing calconcarboxylic acid *N*,*N*{1-(2-hydroxy-4-sulfo-1-naphthylazo)-2-hydroxy-3-naphthoic acid}, Eriochrome Black T/rhodamine B, Evans blue/rhodamine B, and CBB/Bismark Brown R have been introduced with reported sensitivity comparable or better than CBB in SDS-PAGE gels. A very useful general protein stain that also displays group-specific staining is Stains-all^{TM}, a cationic carbocyanine dye, which stains sialoglycoproteins and phosphoproteins blue and almost all other proteins red. **Table 1** summarizes some of the more commonly used organic dyes for protein staining.

Silver Staining

For most applications, visualization of proteins with CBB is sufficiently sensitive. However, if one is interested in determining the absolute purity or trace amounts of a protein then more sensitive techniques must be utilized. To accomplish this, Merril developed an ultrasensitive silver staining procedure based on photographic principles. Silver staining is upward to 100-fold more sensitive than CBB-R with sensitivity comparable to, or greater than, autoradiography, for selected polypeptides. It should be noted, however, that many proteins respond differently to silver staining. Some proteins may not stain at all, so sensitivity values for silver staining may vary from protein to protein.

Silver staining or silver shadowing procedures can be divided into three basic catagories: diamine or ammoniacal silver stains; chemically developed nondiamine type; and photoreduction silver stains. The diamine or ammoniacal silver stains utilize ammonium hydroxide to form soluble silver–diamine complexes and proteins are visualized by acidification, usually with citric acid in the presence of formaldehyde. Diamine stains are rather time-consuming (overnight fixation and 6 h staining) but are particularly good for the staining of gels thicker than 1 mm. The nondiamine chemical development stains are generally more rapid than the diamine stains but display higher backgrounds and are best suited for gels 1 mm or thinner. Image development of nondiamine stains occurs as a result of selective reduction of silver ions to elemental metallic silver by formaldehyde under alkaline pH. The photoreduction silver stains are the most rapid, allowing the visualization of protein patterns within 10 min after electrophoretic separation; however, they are the least sensitive of the silver-based staining methods.

While most proteins stain monochromatically with silver, yielding brown or black spots and bands, certain silver stains can produce varying shades of black, blue brown, red and yellow and the staining of individual proteins appears to be group-specific. Lipoproteins tend to stain blue while glycoproteins appear yellowish-brown, or red. Colour formation has been shown to be highly dependent on the size and distribution of the silver grains within the gel as well as the refractive index of the gel and standardized colour-based silver staining kits are commerically available.

Reverse Staining

In contrast to the positive-staining procedure described above, alternative but generally less sensitive staining procedures based on the formation of insoluble metal (zinc, copper and potassium) salts have also been described. These methods, commonly

Table 1 Reagents useful for protein visualization on gels and membrane supports

Stain/dye	*Sensitivity*	*Gel*	*Membranes*			*Comments*
			NC	*PVDF*	*Nylon*	
Organic dyes						
Coomassie Brilliant Blue (CBB-R) and (CBB-G)	100–1000 ng	yes	yes	yes	no	Permanent, convenient to use, sensitive, anionic, relatively high background (CBB-R), (>1 h)[a]
Ponceau S	1–2 μg	yes	yes	yes	no	Reversible, low background, rapid staining/destaining (<2 min)
Amido Black	1–2 μg	yes	yes	yes	no	Permanent, low background, anionic stain, stains histones and oroso-mucoids (>30 min)
India Ink (colloidal carbon)	80–100 ng	no	yes	yes	yes	Permanent, very sensitive, low background, sensitivity dependent upon source/lot of ink (>2 h), useful for charged nylon membranes
Fast Green FCF	1–2 μg	yes	yes	yes	no	Permanent, linearity of staining>CBB, does not bind IEF ampholytes, stains histones (30 min)
Stains-All™	1–2 μg	yes	yes	yes	no	General protein and nucleic acid stain, stains RNA (bluish purple), DNA (blue), most proteins (red) sialoglycoproteins and phosphoproteins (blue)
Periodic acid–Schiff (PAS)	25–100 ng	yes	yes	yes	no	General protein and glycoprotein stain. Silver enhancement increases sensitivity (~0.4 ng)
Procion Blue RS	1–2 μg	yes	yes	yes	no	Anionic, hydrophobic dye, occasionally used
Alcian Blue	500–700 ng	yes	yes	yes	no	Useful for staining glycosaminoglycan
Eosin Y	10 ng	yes	yes	yes	no	General protein stain, stains sialoglycoproteins
Congo Red	500 ng	yes	yes	yes	no	Low background, rapid (5 min), anionic stain
Fluorescent stains						
Dansyl chloride	100–200 ng	yes	yes	yes	no	Stains all types of proteins, including proteoglycans
SYPRO Red/Orange	100 ng	yes	no	no	no	Exhibits a greater linearity of staining than CBB, SDS-PAGE only, does not require protein fixation, will not stain nucleic acids
Fluorescamine	6–10 ng	yes	yes	yes	no	Neither fluorescamine nor its degradation products are fluoresecent, low backgrounds
Fluorescein isothiocyanate	50 ng	yes	yes	yes	no	Rapid, can be used as pre-electrophoretic tag
Metal salt complexes						
Silver	1–3 ng	yes	yes	yes	no	Permanent, very sensitive, stains nucleic acids, hundreds of modifications, time-consuming
Colloidal gold (Aurodye™)	1–3 ng	yes	yes	yes	no	Permanent, very sensitive, silver enhancement increases sensitivity
Potassium chloride	10–100 ng	yes	no	no	no	Reversible, SDS-PAGE only, negative stain, whiter bands on opaque background
Iron (Ferridye™)	1–3 ng	yes	yes	yes	yes	Permanent, useful for nylon membranes
Copper (iodide/chloride)	10–100 ng	yes	no	no	no	Reversible, rapid (5 min), SDS-PAGE only, negative stain, clear bands on opaque background
Copper phthalocyanine 3,4′,4″,4‴ tetrasulfonic acid	10–100 ng	yes	yes	yes	no	Reversible, rapid (<2 min), sensitive
Zinc/imidazole SDS	10–100 ng	yes	no	no	no	Reversible, metal chelate stain, SDS-PAGE only, negative stain, clear bands on opaque background, does not require protein fixation, protein eluted from gels with high efficiency (>90%)

[a]Staining/destaining time. NC, Nitrocellulose; PVDF, polyvinylidene fluoride.

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referred to as negative or reversible staining, are limited to SDS-containing gels and produce a semi-opaque background on the gel surface where proteins are detected as whiter or transparent bands or spots when viewed against a black background or when back-lit. Staining procedures are rapid (within 15 min), display intermediate sensitivity between that of CBB and silver staining, and since minimal protein fixation is required, proteins are readily eluted from gels (>90%) for biochemical characterization including Western immunoblotting, amino acid composition analysis and Edman N-terminal amino acid microsequencing.

Fluorescent Protein Labelling

Fluorescent methods for protein visualization are extremely sensitive but are less frequently used than the CBB/silver staining protocols due to their relative complexity (e.g. they require ultraviolet illumination for protein visualization, and fluorescence signal intensities diminish with time) and increased cost. Proteins can be tagged either pre- or post-electrophetically with fluorescent sensitive dye(s) via covalent interaction of the dye with terminal -NH_2 groups of the proteins. Fluorophores most commonly utilized to label proteins prior to electrophoresis include dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride), fluorescamine (4-phenylspiro(furan-2[^{3}H]-1′-phthalan-3,3′ dione), MDPF (2-methoxy-2,4-diphenyl-3-(^{2}H)-furanone), DACM (*N*-(7-dimethylamino-4-methylcoumarinyl)maleimide) and OPA (*o*-phthaldialdehyde). Although dansyl chloride was the first fluorescent dye used for pre-electrophoretic labelling of proteins, fluorescamine has found increasing use since neither fluorescamine nor its hydrolysis products are fluorescent. Fluorescamine is capable of detecting as little as 6 ng of myoglobin while MDPF, which is 2.5 times more sensitive than fluorescamine, is very useful for quantitative applications and displays a linear staining response from 1 to 500 ng.

Proteins can also be detected post-electrophoretically using fluorescent reagents such as ANS (1-anilinonaphthalene-8-sulfonate), Bis-ANS, fluorescamine, *p*-hydrazinoacridine and OPA. Since labelling is usually performed under nondenaturing conditions, these reagents can be used quite advantageously for the rapid detection of proteins during preparative electrophoresis. Generalized procedures for both pre- and post-labelling with fluorescent dyes have appeared in reviews by Hames and Rickwood and Merril. Table 1 briefly summarizes the major metal-based and fluorescent dyes used to stain proteins on polyacrylamide gels.

Autoradiographic Detection

Labelling of proteins either prior to or post-electrophoretically using radioactive isotopes remains the most sensitive method for protein detection. Individual radiolabelled protein bands or spots are usually detected in one of three ways: liquid scintillation counting, autoradiography and fluorography. Modifications to facilitate the detection of proteins expressed at very low concentrations (e.g. transcription factors, cytokines, single copy gene products) or proteins labelled with low energy β-particle-emitting radioisotopes, such as [^{3}H], include indirect autoradiography which utilizes intensifying screens for signal enhancement and fluorography.

Autoradiography

In autoradiography dried polyacrylamide gels containing radiolabelled proteins are placed in direct contact with the appropriate X-ray film (e.g. Kodak X-Omat AR, Kodak SB-5, Kodak BMS2, Kodal BMR2, Fuji RX) where radioactive emissions (β-particles and/or β/γ-radiation) react with the silver halides in the film emulsion, resulting in the formation of elemental silver atoms which are visualized following photographic development of the films. [^{14}C]-, [^{35}S]-, [^{32}P]-, [^{125}I]- and [^{131}I]-labelled proteins are readily detected using direct autoradiography while [^{3}H]-labelled proteins are very weakly detected due to severe quenching of their low energy β-emissions by the polyacrylamide gel matrix.

Fluorography and Indirect Autoradiography

To enhance the detection of low abundance proteins and proteins labelled with low energy β-type emitters, fluorographic and indirect autoradiographic methodologies have been developed. Both procedures provide enhanced autoradiographic imaging of low to medium energy β-particle emitters (^{3}H, ^{14}C or ^{35}S) and involve the conversion of the emitted energy from the respective isotopes to photons of visible light, which become the predominant exposing radiation. This is accomplished either by the incorporation of an organic scintillator, PPO (2,5-diphenoxyoxazole), directly into the polyacrylamide gel matrix prior to fixation, drying and exposure to film (fluorography) or by the use of calcium tungstate X-ray intensifying screens (indirect autoradiography), as originally developed by Bonner and Laskey and Mills, respectively. For optimal sensitivity, film exposure utilizing X-ray intensifying screens such as Kodak X-OMATIC and Dupont Cronex Lightning Plus or Cronex Quanta II should be performed at low temperatures (-70°C) to stabilize latent image formation. This results in up to a 30–40-fold increase in the

detection of [^{125}I] and 8–10-fold increase in sensitivity to [^{32}P]. Additional rare earths (europium-activated barium fluorochloride or terbium-activated mixtures of lanthanium oxysulfide and gadolinium oxysulfide) are available and these appear to be more efficient than calcium tungstate for [^{32}P] detection but result in higher background film darkening. The sensitivity of fluorography can be further increased by pre-flashing (< 1 ms) or hypersensitizing the film before main exposure. This step has the added benefit of correcting the so-called toeing effect or nonlinear relationship between the radioactivity in a sample to the absorbance of the film image, thus permitting quantitative measurements. If an autoradiogram or fluorogram is too faint then it is possible to intensify the images up to 10-fold by incubation of the X-ray film in [^{35}S]-thiourea which complexes with the silver ions in the film to form silver [^{35}S] sulfide.

Storage Phosphor Imaging

Two of the most serious limitations to the use of X-ray film for the visualization of isotopically labelled proteins are relative insensitivity to low energy β-radiation and a nonlinear, limited dynamic range of film darkening to radiation exposure. An alternative to X-ray film for the detection and quantification of autoradiography is photostimulable storage phosphor imaging. Storage phosphor imaging exhibits a dynamic exposure range of more than five orders of magnitude (100 000 : 1 versus 300 : 1 for X-ray film) and a 10–250-fold greater sensitivity than autoradiography to β-emissions. Dried gels containing radiolabelled proteins are exposed to imaging screens composed of a thin layer of BaFBr : Eu^{+2} crystals in an organic binder in the same manner in which X-ray film is exposed. Incident radiation (β-particles, γ-rays, X-rays) from labelled proteins induces excitation of the Eu^{+2} ions in the phosphor complex which stores this energy as a latent image. The latent images are scanned with a helium–neon laser which releases the stored energy as blue photons and the intensity of luminescence is quantitated. [^{14}C], [^{35}S], [^{32}P], [^{33}P], [^{125}I] and [^{131}I] are readily detected and quantitated.

Labelling of Proteins with Radioactive Isotopes

The most commonly used isotopes include [^{14}C], [^{35}S], [^{32}P], [^{3}H] and [^{125}I], although metal isotopes such as [^{59}Fe], [^{45}Ca], [^{63}Ni] and [^{75}Se] have been used to identify iron, calcium and nickel binding proteins and Se-cysteine-containing proteins, respectively. Whereas [^{32}P]-orthophosphate has been used for the introduction of radioactive phosphate groups into proteins, the substitution of [^{33}P] for [^{32}P] has gained in popularity because of significantly increased resolution band sharpness as well as increased safety factors afforded by the lower energy [^{33}P] emitter as compared to [^{32}P].

In vitro metabolic labelling of cells or tissue sections in short term culture by incorporation of isotopically labelled amino acid(s) precursor molecules during the cellular growth phase is usually performed using either [^{3}H]-leucine or [^{35}S]-methionine/cysteine. The use of [^{35}S]- is favoured because of its higher energy β-emitter potential (0.167 vs. 0.018 MeV), higher specific activity (>1000 vs. 50 Ci mmol^{-1}) and lower cost than [^{3}H]-labelled molecules. The extent of incorporation of [^{35}S]-methionine is, however, dependent upon methionine content of the individual proteins and proteins lacking methionine would be undetected using [^{35}S]-methionine labelling. This problem has been cricumvented by labelling with [^{14}C] amino acid mixtures, although this method is less favoured due to significantly higher cost and lower specific activity (50 mCi mmol^{-1}) of [^{14}C] versus [^{35}S]. Proteins which are post-translationally modified via glycosylation can be labelled with [^{3}H]/[^{14}C]-galactose, mannose, *N*-acetyl-glucose and galactoseamine (carbohydrates), respectively, while lipoproteins and certain membrane-associated polypeptides can be labelled with [^{3}H]/[^{14}C] palmitate and myrisoylate.

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Radioactive Stains

The use of radioactive stains for the *in situ* detection of proteins has found limited applications because of the availability of relatively few radiolabelled reagents. [^{59}Fe]-ferrous bathophenanthroline has been used to label radioactively a series of protein markers in polyacrylamide gels post-electrophoretically using simple staining and destaining procedures.

Protein Blotting

One the major advances in the analysis of proteins has been the development of post-electrophoretic techniques for the transfer and immobilization of proteins from the polyacrylamide gel matrix to thin support membranes. Originally based on DNA Southern and RNA Northern blotting principles, protein-blotting protocols were similarly developed by Towbin and co-workers utilizing the electrophoretic elution of proteins separated by PAGE to nitrocellulose (NC) sheets. The major advantage of protein electroblotting is that separated proteins are transferred from the gel matrix, where their access to detection reagents is severely hindered, to the surface of a membrane where the protein molecules are readily accessible. Although protein blotting has traditionally been associated with the immunodetection of

antigen–antibody complexes (Western immunoblotting), blotted proteins are amenable to analysis and characterization via a multitude of visualization and overlay techniques. These include general protein staining and autoradiography, group-specific ligand binding, receptor–ligand interaction, enzymic activity determination as well as amino acid composition and primary amino acid sequence analysis of individual spots or bands. A single protein blot offers numerous advantages not afforded by polyacrylamide gels. It is easily handled and manipulated, can be stored for up to 1 year and the blot can be used for multiple successive analyses. Once a signal has been obtained and recorded, the blot can be erased by removing the probe or stain while retaining the original protein pattern on the membrane and the blot reprobed. Protein blotting has been said to add a second and third dimension to 1D and 2D-PAGE, respectively.

Various types of membranes have been used for protein blotting and immobilization. Nylon and NC sheets (thin film on cellulose esterified with nitric acid) were first used but recently different types of polyvinylidene fluoride (PVDF) membranes have been introduced. A detailed description of protein-blotting methodology, including the advantages and disadvantages of the various blotting membranes, is beyond the scope of this article (see Further Reading).

Immunological Detection

Immunological or group-specific detection of protein(s) on blotted membranes is far and away the most utilized method for protein detection but is limited by the available of appropriate antibodies/ligands. Following protein transfer, membranes are incubated with dilute protein solutions (e.g. bovine serum albumin, gelatin or instant nonfat dry milk) to block nonspecific binding sites on the membranes. The membrane-bound proteins are incubated with either monoclonal or polyclonal antibodies directed against specific target antigens or group-specific ligands (e.g. Concanavilin A for the detection of glycoproteins, ^{59}Fe and ^{45}Ca to detect iron- and calcium-binding proteins, and [^{32}P]-labelled DNA to detect DNA binding proteins). NC and PVDF membranes are most frequently used since nylon membranes with their intrinsically higher binding affinity for proteins are more difficult to block. If antibodies are used, then blots are incubated with a second antibody that has been directed toward the primary antibody and has been tagged with a reporter label. Typically, the second anti-species antibody may be radiolabelled (^{125}I) or conjugated with an enzyme (e.g. horseradish peroxidase (HRP), alkaline phosphatase (AP) or β-galactosidase). The resulting ((antigen–1° antibody)–2° antibody) complex is detected either autoradiographically or colorimetrically using an appropriate chromogenic substrate. The advantages of enzyme-conjugated antibodies are ease of handling and storage and rapid development of colour (min vs. day). Sensitivity is usually in the range of 0.1–10 ng of antigen/band. While this sensitivity is approximately 10–100-fold less sensitive than autoradiographic or fluorographic detection, it is possible to achieve a similar level of enhanced sensitivity using peroxidase–antiperoxidase (PAP) sandwiching.

Recently, modifications have been developed for the use of light emission (luminescence) as an end point for protein–antibody detection on membranes. Although substrates which produce colour complexes exist for both AP and HRP, the HRP luminescent system, in which blue light is generated by the HRP-mediated oxidation of luminol, is the most sensitive system. This system, commonly known as the ECL (enhanced chemiluminescence) Western blotting system, provides excellent signal-to-noise ratio and is extremely rapid and sensitive.

In the basic (nonenhanced) chemiluminescent reaction, HRP is used to oxidize a peracid, resulting in a raised oxidation state of the haem Fe in HRP. Relaxation of this excited state to initial (ground) state occurs in a two-step process. At each relaxation a luminol radical is formed and, as each radical decays, light is emitted. However, in the ECL reaction, an enhancer molecule is added which reacts with the haem Fe in place of the luminol molecule, resulting in the formation of enhancer radicals which themselves react to produce luminol radicals and light is emitted. The enhancer molecules increase light emission greater than 1000-fold over luminol alone. Light emission on membranes rises rapidly over the first 5 min, remains at maximum for 15 min, and then declines with a $t_{1/2}$ of 60 min. Typical exposures for ECL are of the order of a few seconds to minutes and are capable of detecting 1 pg or less of protein.

Total Protein Staining

Blotted membranes can be stained with many of the general protein stains used for polyacrylamide gels including Amido Black, CBB, Ponceau S, Fast Green and India ink (Table 1). Amido Black and Ponceau S are preferred to CBB-R because stained membranes can be destained quickly to leave very low backgrounds, whereas CBB-R gives higher backgrounds. India ink (colloidal carbon) is the most sensitive of the above dyes and can detect as little as 80 ng of protein but staining sensitivity is highly dependent upon dye source and lot. Silver staining is also possible as well as the use of colloidal gold and iron sol stains. Both silver and gold stains can detect as little as 1–5 ng protein on NC and PVDF membranes and

the sensitivity of gold stain can be further enhanced by incubation with a silver lactate solution such that as little as 400 pg of protein per band can be detected. Although nylon or charged nylon membranes possess the greatest protein-binding capacitites (450 vs. 80 $\mu g\,cm^{-2}$ (NC/PVDF)), staining of nylon membranes is very problematic. Anionic organic dyes as well as colloidal gold and silver are not useful for staining nylon membranes due to extremely high backgrounds. However, colloidal sols are especially useful for the detection of proteins on nylon membranes. On nylon membranes the positively charged colloidal iron particles bind to negatively charged SDS-denatured proteins and protein staining can be intensified using potassium ferricyanide, which gives deep blue-stained bands with low backgrounds. India ink and a modified silver stain have been reported to have been used to stain charged nylon membranes.

A less frequent, but none the less useful method for the visualization of protein bands on NC and charged nylon membranes involves protein iodination *in situ* with chloramine T/potassium iodide, followed by formation of a purple complex between the bound iodine and starch.

Autoradiographic Detection

Electroblotting of proteins radiolabelled with ^{14}C or ^{35}S permits more efficient autoradiography since the gel matrix is no longer present to quench the β-emissions. The minimum level of ^{14}C or ^{35}S that can be detected in 24 h is about 400 $dpm\,cm^{-2}$. While fluorography is necessary to detect ^{3}H on polyacrylamide gels, ^{3}H exposure can be detected directly on electroblots using autoradiography, although 2×10^4 $dpm\,cm^{-2}$ is required for detection in 24 h. The efficiency of detection for all isotopes is enhanced if fluorography is employed (100 dpm and 500 $dpm\,cm^{-2}$ for $^{14}C/^{35}S$ and ^{3}H, respectively).

Future Developments

PAGE, in particular 2D-PAGE, remains the method of choice for the separation of complex protein mixtures. This has necessitated the development of highly sensitive protein visualization protocols incorporating both nonradioactive and radioisotopic imaging methodologies. The development of methods for the transfer of polypeptides from gels to membranes where they are readily accessible to react with stains, specific antibodies, group-specific ligands and detailed structural characterization, including amino acid microsequencing and mass spectral analysis, has permitted the identification of previously unidentified proteins. Further developments are likely to take place in low background staining polyacrylamide formulations and modified membrane support matrices in which proteins may be bound either covalently or which form reversible covalent bonds. Such proteins can be easily and selectively eluted for more detailed biochemical studies. Future advances are likely to take place in the development of more sensitive and group-specific dyes/stains and increased speed and sensitivity of detection systems such as the enhanced bioluminescent and chemiluminescent systems, as well as the development of faster and more sensitive photographic detection film.

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Detectors for Capillary Electrophoresis

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Detection is a particularly critical issue in capillary electrophoresis (CE) because of the extremely small cell volumes available. Considerable effort has gone into overcoming this limitation and a bewildering